

Atty's Docket No: 101215-18
Hillebrand, et al., DeclarationIN THE UNITED STATES PATENT AND TRADEMARK OFFICE

SERIAL NO.	09/454,740
APPLICANT	Timo HILLEBRAND et al.
FILED	December 6, 1999
EXAMINER	A. Chakrabarti
ART UNIT	1634
FOR	FORMULATIONS AND METHOD FOR ISOLATING NUCLEIC ACIDS FROM OPTIONAL COMPLEX STARTING MATERIALS AND SUBSEQUENT COMPLEX GENE ANALYSIS

DECLARATION UNDER 37 C.F.R. § 1.132Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

I, the undersigned declarant, hereby declare as follows:

I am a named inventor in the above-referenced application.

I am a German citizen and reside in Berlin, Germany;

I and the co-inventors conducted the experiments set forth in detail in the attached description, and I submit this declaration in support of the above-referenced application.

1. We have conducted experiments directed toward finding more rapid and cost-effective methods for purifying nucleic acids from various types of mixtures. For example, reaction mixtures that include previously purified DNA and various enzymes (i.e., restriction endonucleases, polymerases, etc) or cell and tissue lysates. Unexpectedly, we found that nucleic acids could bind to insoluble supports in the absence of chaotropic salt components. We have also been quite surprised to ascertain the scope of anti-chaotropic salt

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components that create an environment conducive to such binding.

2. The basic protocol used in the experiments presented in the attached exhibit shows that genomic DNA can be easily isolated from 10mg of mouse liver. The liver lysates were prepared in the buffers described on either page 2 or 4. Preparing the lysate is optionally facilitated by the addition of Proteinase K, a proteolytic enzyme that helps to lyse the proteinaceous debris in the lysate. The mixture was loaded onto a spin filter column and centrifuged. The lysate material passing through the filter column was removed and the filter column was washed twice. The filter was then washed with elution buffer and centrifuged. The elution buffer containing the eluted DNA was collected and analyzed by agarose gel electrophoresis.

3. The results obtained surprisingly indicated that nucleic acid binding to insoluble supports is clearly demonstrable in the complete absence of any chaotropic salt component. Equally surprising is the fact that this was observed whether the binding step was performed using previously purified nucleic acids (not shown herein) or whether it was performed directly from a crude lysate of cells, tissue, or other complex biological material.

4. Experiment I, beginning on page 2 of the accompanying exhibit makes three relevant points. First, lane A of the gel (page 3) indicates that Tris and proteinase K alone do not allow binding of genomic DNA to the support (spin column). The second point is NH_4Cl at concentrations above 1M provided excellent binding of the DNA. See lanes 5-7. And third, lower concentrations, e.g., 0.1M, did not result in appreciable binding to the filter columns. Lane 4.

5. A virtually identical DNA binding profile was demonstrable by substituting KCl for NH_4Cl at the same concentrations. Specifically, there was no appreciable binding at 0.1M KCl (lane 12). However, substantial binding was clearly demonstrable at from 1.0M to 2.0M KCl, lanes 13-15. Again, in the absence of any salt, no binding occurred.

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Thus, the antichaotropic salts NH_4Cl and KCl promote binding of nucleic acid in a concentration dependent manner. It is not simply the absence of a chaotropic salt that promotes the binding activity.

6. Further, we tested the efficacy of the lysis/binding composition provided in the reference by Anderson et al. (0.1M NaCl) against the claimed embodiment containing 1M NH_4Cl . In Experiment II, beginning on page 8 of the exhibit, we demonstrate that Anderson's buffer conditions are not conducive for binding of genomic DNA present in a crude tissue lysate, to the insoluble support. See lanes 6 and 7. In contrast, 1M NH_4Cl allowed easily detectable levels of genomic DNA to be bound and eluted from the spin filter column; in lanes 3 and 4.

7. In conclusion, the unexpectedly surprising finding that nucleic acid binding to insoluble supports does not require chaotropic salts clearly comprise a patentable contribution to the molecular biological arts. In addition, it is not merely the absence of a chaotropic salt component, but the presence of at least one antichaotropic salt that promotes the binding. Anti-chaotropic salt components are inexpensive, relatively non-hazardous strongly promote nucleic acid binding activity to insoluble supports, to affect a rapid isolation.

All statements made herein on knowledge are true, and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Dec. 27, 2002


Peter Bendzko

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Experiment I

Isolation of genomic DNA from complex tissue sample (mouse liver; 10 mg) using lysis/binding buffer formula containing an antichaotropic salt component (based on monovalent cations) versus a lysis/binding buffer formula containing no antichaotropic salt

1. Buffer compositions:

Lysis/Binding Buffer A: 5 mM Tris HCl (7.5); Proteinase K

Lysis/Binding Buffer B1: 5 mM Tris HCl (7.5); Proteinase K; 0.1 M NH₄Cl

Lysis/Binding Buffer B2: 5 mM Tris HCl (7.5); Proteinase K; 1 M NH₄Cl

Lysis/Binding Buffer B3: 5 mM Tris HCl (7.5); Proteinase K; 1.5 M NH₄Cl

Lysis/Binding Buffer B4: 5 mM Tris HCl (7.5); Proteinase K; 2.0 M NH₄Cl

Lysis/Binding Buffer C1: 5 mM Tris HCl (7.5); Proteinase K; 0.1 M KCl

Lysis/Binding Buffer C2: 5 mM Tris HCl (7.5); Proteinase K; 1 M KCl

Lysis/Binding Buffer C3: 5 mM Tris HCl (7.5); Proteinase K; 1.5 M KCl

Lysis/Binding Buffer C4: 5 mM Tris HCl (7.5); Proteinase K; 2.0 M KCl

2. Procedure

1. Lysis of starting material in 400 µl Lysis/Binding Buffer at 52°C.
2. Transfer of the lysis mixture into spin filter columns containig glass material; centrifugation for 1 minute.
3. Addition of 750 µl ethanol containing Wash Buffer and centrifugation for 1 minute.
4. Second washing step. Finally drying the spin filter by centrifugation for 3 minutes.
5. Addition of 200 µl od Elution Buffer (10 mM Tris HCl) and centrifugation for 1 minutes to elute the DNA.

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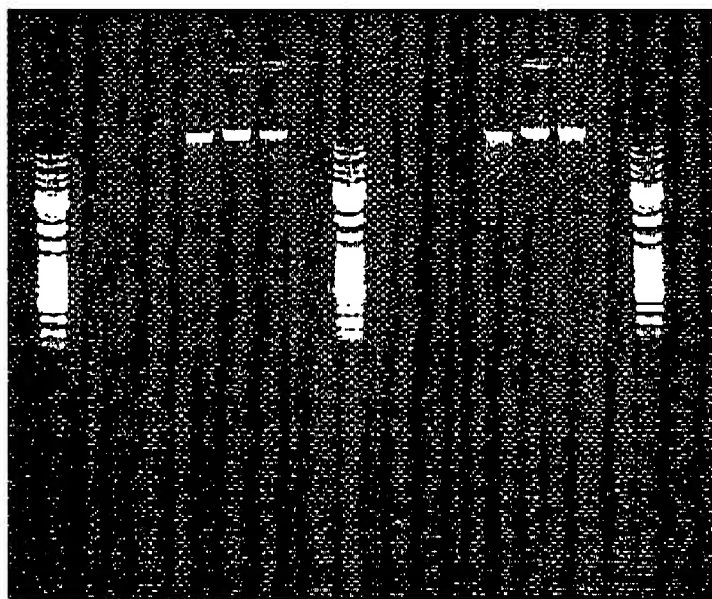
3. Results

Gel electrophoretic analysis of isolated DNA on 0.8 % TAE agarose gel. Each lane was loaded with 20 µl of the DNA eluted from the spin column.

lanes:

- 1: DNA ladder
- 2: empty
- 3: Lysis Binding Buffer A
- 4: Lysis Binding Buffer B1
- 5: Lysis Binding Buffer B2
- 6: Lysis Binding Buffer B3
- 7: Lysis Binding Buffer B4
- 8: empty
- 9: DNA ladder
- 10: empty
- 11: Lysis Binding Buffer A
- 12: Lysis Binding Buffer C1
- 13: Lysis Binding Buffer C2
- 14: Lysis Binding Buffer C3
- 15: Lysis Binding Buffer C4
- 16: empty
- 17: DNA ladder

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17



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Experiment II

Isolation of genomic DNA from complex tissue sample (mouse liver; 10 mg) using lysis/binding buffer formula containing an antichaotropic salt component versus the buffer formula from ANDERSON

1. Buffer compositions:

Lysis/Binding Buffer B2: 5 mM Tris HCl (7.5); Proteinase K; 1 M NH₄Cl

Lysis Buffer from Anderson

2. Procedure

1. Lysis of starting material in 400 µl Lysis/Binding Buffer B2 at 52°C or Lysis Buffer from Anderson (Lysis by 1 h permanent vortexing using Inviprep Gyrator).
2. Transfer of the lysis mixture into spin filter columns containig glass material; centrifugation for 1 minute.
3. Addition of 750 µl ethanol containing Wash Buffer and centrifugation for 1 minute.
4. Second washing step. Finally drying the spin filter by centrifugation for 3 minutes.
5. Addition of 200 µl od Elution Buffer (10 mM Tris HCl) and centrifugation for 1 minutes to elute the DNA.

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3. Results

Gel electrophoretic analysis of isolated DNA on 0.8 % TAE agarose gel. Each lane was loaded with 20 µl of the DNA eluted from the spin column.

lanes:

- 1: DNA ladder
- 2: empty
- 3: Lysis Binding Buffer B2
- 4: Lysis Binding Buffer B2
- 5: empty
- 6: Lysis Buffer ANDERSON
- 7: Lysis Buffer ANDERSON

1 2 3 4 5 6 7

